perniciosus (Comst.) tripled the oxygen consumption of plants. Similarly, LADD and RAWLINS[3] also noted increases in respiration in the potato plant as a result of the feeding of the leafhopper, *Empoasca fabae* (Harr.)

The increased respiratory rate in plants always coincides with the increased protein synthesis[4]. This has proved true in the case of the susceptible variety Dominica, where jassid injury has invariably been followed by increase of total nitrogen content and total amino acid content[5]. This phenomenon may be explained in the light of the results of SPIEGELMAN et al.[6]. The required energy for the increased rate of protein synthesis is provided by enhanced rate of respiration. STEWARD et al.[7] and JAMES[8] also reported that increased respiration results in increased protein synthesis in plants.

In the plants of Dominica variety injured by hopperburn, a high accumulation of sucrose was observed[5]. The sucrose content and respiration, sucrose content and total nitrogen, and total nitrogen and respiration were all positively correlated. This is in conformity with the observations made by RICHARDS[9] on barley.

Zusammenfassung. Die Saugtätigkeit der Jasside *Empoasca flavescens* (F.) führte zu einer um 44% gesteigerten Respiration von geschädigten, anfälligen Rizinuspflanzen (*Ricinus communis* L.) im Vergleich zu 11.6% und 3.7% bei Pflanzen einer toleranten und resistenten Sorte. Diese Zunahme war bei der anfälligen Sorte verknüpft mit einer gesteigerten Anhäufung von Rohrzucker, Gesamtstickstoff und Gesamtaminosäuregehalt.

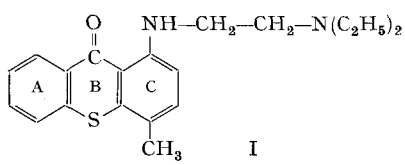
S. JAYARAJ[10,11]

Faculty of Entomology, Agricultural College and Research Institute, Coimbatore (Madras, India), January 24, 1966.

[3] T. L. LADD JR. and W. A. RAWLINS, J. econ. Ent. *58*, 623 (1965).
[4] B. S. MEYER and D. B. ANDERSON, *Plant Physiology* (D. Van Nostrand Co., London 1959).
[5] S. JAYARAJ, Doctoral Thesis, University of Madras (1964), unpublished.
[6] S. SPIEGELMAN, J. M. REINER, and R. COHNBERG, J. gen. Physiol. *31*, 27 (1947).
[7] F. C. STEWARD, W. A. PRESTON, and T. K. RAMAMURTHI, Ann. Bot., Lond. *7*, 221 (1943).
[8] W. O. JAMES, *Plant Respiration* (Oxford Press, London 1953).
[9] F. J. RICHARDS, Ann. Bot., Lond. *2*, 491 (1938).
[10] This study formed part of the Doctoral Thesis submitted to the University of Madras and financial support was given by the Council of Scientific and Industrial Research, Government of India, New Delhi. Grateful appreciation is extended to Prof. A. R. SESHADRI, Agricultural College and Research Institute, Coimbatore, India and Prof. R. H. PAINTER, Kansas State University, Kansas, U.S.A. for their valuable suggestions made during the course of the study.
[11] Present address: Tropeninstitut, Abt. Phytopathologie und Angewandte Entomologie, Justus Liebig-Universität, Giessen (West Germany).

Antagonism of Heterocyclic Sulphonamides to the Schistosomicidal Effect of Lucanthone

The schistosomicidal[1] and antineoplastic[2] activity of lucanthone (Miracil D) (I) lies in ring C, where a methyl group is located in a position para to a substituted amino, i.e. a *p*-toluidine system. This reactive system, resulting from the high electron density in ring B, was found to undergo oxidation with hydrogen peroxide through the catalytic effect of peroxidase. Ring C thus became quinoid and was accompanied by the oxidation of the sulphur atom to the sulphone[3]. Other reactions have confirmed the fact that the position of the methyl group makes it very labile[4].



I

Heterocyclic sulphonamides have been found to inhibit the action of peroxidase on the oxidation of aromatic amines by the system peroxidase-hydrogen peroxide[5]. Also, the oxidation of *p*-aminobenzoic acid by peroxidase is inhibited by the presence of sulphonamide[6]. Further, heterocyclic sulphonamides inhibit the antitumour effect of colchicine[7].

These facts induced us to investigate the effect of heterocyclic sulphonamides on the schistosomicidal activity of lucanthone. Three members of this group were chosen, namely sulphathiazole, sulphamethazine and sulphadiazine. Experiments were carried out on mice infected with *Schistosoma mansoni*. In vitro studies will be reported later.

The curative dose of lucanthone is 5 mg/kg twice daily[8]. In our experiments, 20 mg/kg twice daily was administered to infected mice; some of which were treated with lucanthone alone, while the others, divided into 3 subgroups, were administered sulphamethazine, sulphadiazine and sulphathiazole in solution, in an equimolecular dose to lucanthone.

Experimental. Mice weighing between 20 and 35 g were exposed to infection with *Schistosoma mansoni*, using cercaria liberated from various snails. More than one snail was used as a source of infection, since it had been noticed that usually infection originating from one snail gave a unisexual adult infection. For infection, each animal was partially submerged in a 1 l beaker covered with wire

[1] H. MAUSS, H. KOLLING, and R. GONNERT, Medizin Chem. *5*, 185 (1956).
[2] E. BLANZ and F. FRENCH, J. med. Chem. *6*, 185 (1963).
[3] I. NABIH and M. EL SHEIKH, Abstracts of Papers, 150th Meeting, American Chemical Society, Atlantic City, N.J., September 1965.
[4] I. NABIH and M. EL SHEIKH, J. pharm. Sci. *54*, 1696 (1965).
[5] P. ZAMBONI, Atti Soc. lomb. Sci. med. biol. *3*, 322 (1948).
[6] F. LIPMANN, J. biol. Chem. *139*, 977 (1941).
[7] R. BAUCH, Naturwissenschaften *33*, 25 (1946).
[8] L. GOODMAN and A. GILMAN, *Pharmacological Bases of Therapeutics* (The Macmillan Company, New York 1958), p. 1149.

gauze and containing 200 ml of water at 30–40 °C contaminated with approximately 200 cercaria. Exposure was for 3 h. After 6 weeks, 24 h stools were examined for positive infection through an egg count. Each mouse was kept separate. Mice producing stools containing living, hatchable ova were isolated. The hatchability was examined, through the addition of an excess of warm water to the egg concentrate obtained through centrifugation. 25 infected mice were divided into 3 groups: (A) 5 were kept as controls. (B) 5 were given lucanthone (*M* weight 370) in a dose of 20 mg/kg twice daily at a 6 h interval from a water solution (100 mg%) for 15 days. (C) 15 were subdivided into 3 subgroups, each containing 5 mice. Each mouse was administered 20 mg/kg lucanthone, followed by 15 mg/kg of sulphamethazine for the first group, 13.6 mg/kg sulphadiazine for the second and 13.5 mg/kg sulphathiazole for the third. The combined doses were given twice daily at a 6 h interval for 15 days. Follow-up of the egg excretion began right after administration periods for the 3 groups in 24 h stools.

Results and discussion. The A (control) group continued egg excretion for the whole period of examination (4 weeks) parallel to the other 2 groups.

The B group (given lucanthone alone) showed continuous depression of the egg count, and 3–4 weeks after discontinuation of drug administration no more ova could be detected.

The ova count for the members of group C (those administered the sulphonamide together with lucanthone) did not show a depression in the egg count. Living ova were present during the 4-week examination period.

When the animals were sacrificed by decapitation 120 days after administration of either lucanthone alone or lucanthone and sulphonamides, the following observations were recorded. In group A (control), excretion of eggs continued during the period before dissection. Living worms were found in the mesentric venules. In group B, excretion of eggs continued during the entire period before dissection (120 days after the end of administration of drugs). Living worms were found in the liver. This meant an hepatic shift had occurred. In group C, containing animals given lucanthone alone, excretion of eggs ceased 3–4 weeks after stopping drug administration, and after 120 days, when the animals were dissected, in one mouse no worms were found while the other 4 contained dead worms in the liver.

This suggests that lucanthone, with or without sulphonamide, causes hepatic shift of worms from the mesenterics, and the addition of sulphonamide antagonizes the therapeutic action of lucanthone in the liver. These results support our earlier hypothesis that peroxidase is involved in the chemotherapeutic effect of lucanthone[3].

Zusammenfassung. Heterocyclische Sulfonamide, in äquimolekularer Menge an mit *Schistosoma mansoni* infizierte Mäuse verabreicht, heben die Hemmung von Lucanthone auf die Entwicklung des Parasiten auf.

I. Nabih

National Research Centre, Dokky, Cairo (Egypt), January 13, 1966.

Further Observations on Age Differences in the Effects of Formalin on the Canine Brain in vitro

The effects of formalin fixation on the brain of the Macaque, man and small mammals have been studied by several investigators[1–5] and recently reported in a preliminary study on the dog[6]. Fox[7] demonstrated that there is a linear decrease in the percentage weight gain of whole brain after 24 h in 20% formalin from 47.5% at birth to 15% in the young adult dog, and a decrease in total dry matter content in the cerebrum and medulla[6,7]. The present investigation was conducted to ascertain possible age differences in the effect of formalin fixation on the major cortical regions of the canine brain during postnatal growth.

9 dogs at selected ages were deeply anesthetized with intravenous pentobarbital, and following exsanguination by section of carotids and venae cavae the anemic brains were removed and dissected into appropriate regions and treated as follows:

(1) *Age differences in effect of formalin.* The cerebral cortices of 8 dogs aged 1 day, 1, 2, 3, 4, 12, 16 weeks and adult were dissected and approximately 1 g of tissue was taken from the frontal lobe of each hemisphere in coronal section. The tissue was first weighed on a Mettler shadowgraph balance before immersion in 40% formalin (1 g tissue/30 ml). The formalin was changed daily to insure a constant concentration, and tissues weighed after wiping lightly on filter paper on days 1, 4 and 7.

(2) *Edema properties of the developing brain.* 9 subjects aged 1 day, 1, 2, 3, 4, 7, 12 and 16 weeks and adult were studied (8 of these were used from part 1 after frontal lobe dissection). The parieto-temporal region of the cerebrum was removed, and as in the case of the formalin fixation studies, double samples of similar weight from opposite cortical lobes were studied in each specimen. The parieto-temporal region on each side was dissected into 3 segments, and, after weighing, these segments were placed respectively in normal saline (0.9% NaCl) half normal saline and twice normal saline (1 g tissue/30 ml). Weights were taken after 24 h immersion to determine the increase in weight. Results were averaged from double samples of each age group studied, and all specimens were maintained at 28 °C (± 1 °C) in airtight vials throughout these experiments.

The effects of formalin fixation (Figure 1) involve a rapid increase in the weight of the brain during the first day of immersion followed by a rapid decline in weight, so that by the fourth day considerable shrinkage has

[1] H. Kato, Folia anat. jap. *17*, 237 (1939).
[2] J. G. Frontera, Anat. Rec. *133*, 501 (1959).
[3] B. M. L. Underhill, Jl. R. microsc. Soc. *52*, 113 (1932).
[4] J. G. Frontera, J. comp. Neurol. *109*, 417 (1958).
[5] W. J. Krieg, J. comp. Neurol. *91*, 467 (1959).
[6] M. W. Fox, Nature *205*, 1221 (1965).
[7] M. W. Fox, Am. J. vet. Res. *24*, 1240 (1963).